



Draft Genome Sequences of Two *Burkholderia multivorans* Sequential Isolates from a Chronic Lung Infection of a Cystic Fibrosis Patient

Inês N. Silva,^a Pedro M. Santos,^b DLeonilde M. Moreira^{a,c}

Institute for Bioengineering and Biosciences (iBB), Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal^a; Department of Biology, Centro de Biologia Molecular e Ambiental (CBMA), Universidade do Minho, Campus de Gualtar, Braga, Portugal^b; Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal^c

Burkholderia multivorans belongs to the *Burkholderia cepacia* complex, which comprises opportunistic pathogens infecting cystic fibrosis (CF) patients. Here, we report the genome sequences and annotations of two sequential *B. multivorans* clinical isolates (D2095 and D2214) displaying different traits. The differences in the genomic contents of these isolates may provide clues regarding the evolution of *B. multivorans* within the airways of a CF patient.

Received 18 December 2014 Accepted 5 January 2015 Published 12 February 2015

Citation Silva IN, Santos PM, Moreira LM. 2015. Draft genome sequences of two *Burkholderia multivorans* sequential isolates from a chronic lung infection of a cystic fibrosis patient. Genome Announc 3(1):e01531-14. doi:10.1128/genomeA.01531-14.

Copyright © 2015 Silva et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

Address correspondence to Leonilde M. Moreira, Imoreira@tecnico.ulisboa.pt.

he Burkholderia cepacia complex comprises 18 species of closely related betaproteobacteria capable of establishing infections in cystic fibrosis (CF) and immunocompromised patients (1–3). These microorganisms have large genomes and a high gene content, providing them with the ability to rapidly adapt and colonize different niches, like the airways of CF patients (4). Within the B. cepacia complex, the species Burkholderia multivorans is one of the most frequently isolated from CF patients (2). Moreover, B. multivorans undergoes phenotypic changes while chronically colonizing CF airways, and mucoid-to-nonmucoid transitions within CF lungs have been reported (5, 6). A genomic comparison between two B. multivorans sequential isolates that underwent a mucoid-to-nonmucoid morphotype transition within the CF lung is lacking. Here, we report the genome sequences of two isolates, D2095 and D2214, isolated from the same CF patient attending a Vancouver CF clinic. D2095 was isolated from a throat swab in June 2006 and was highly mucoid in yeastextract-mannitol (YEM) solid medium due to exopolysaccharide production, while D2214 was isolated from a sputum sample in November 2006 and displayed a nonmucoid morphotype (5). An extensive phenotypic characterization of these isolates, together with transcriptomic profiling and morphotype stability studies, was performed (7, 8).

Genomic DNA from *B. multivorans* D2095 and D2214 was extracted and purified using the DNeasy blood and tissue kit (Qiagen), according to the manufacturer's instructions. Genomic libraries were prepared using the TruSeq SBS kit version 5 (Illumina), and genome sequencing was performed using Illumina HiSeq 2000 technology, giving rise to paired-end libraries of 2×100 bp, with an insert size of approximately 300 bp (~1,000× coverage). The raw paired-end reads of D2095 and D2214 were assembled using Edena version 3.131028 (9), and the assembly of the genomes was finished using SSPACE Premium version 2.3 (10). For D2095 scaffolding, an additional paired-end library and a mate-pair library were used. Genomic DNA of *B. multivorans*

D2095 was resequenced using Illumina HiSeq 2000 technology, yielding an extra 2×100 -bp paired-end library, with an insert size of 300 bp (\sim 80× coverage), and a 2 × 50 mate-pair library, with an insert size of 5 kb (~70× coverage). The D2095 and D2214 genomes were assembled into 15 and 26 contigs, accounting for 6,668,882 bp and 6,465,997 bp and estimated G+C contents of 67.1% and 67.4%, respectively. The D2214 assembly was more fragmented due to the nonusage of a mate-pair library during the scaffolding process. Both genome drafts were annotated using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline, which revealed that the D2095 isolate possesses 5,952 open reading frames (ORFs), whereas D2214 has 5,782 ORFs. A major genome deletion of 175 kb was identified in D2214, consisting mainly of genes encoding hypothetical proteins. We also identified in the D2214 genome a 37-kb genome duplication of phage-related protein-coding genes. Further comparative genomics of both isolates will bring insights into the mechanisms of virulence/persistence and evolution employed by *B. multivorans* within the airways of a CF patient.

Nucleotide sequence accession numbers. The *B. multivorans* D2095 and D2214 whole-genome shotgun sequence projects were deposited in DDBJ/EMBL/GenBank under the accession numbers JFHP00000000 (D2095) and JFHQ00000000 (D2214). The versions described in this paper are the first versions.

ACKNOWLEDGMENTS

This work was supported by FEDER and the Fundação para a Ciência e a Tecnologia, Portugal (project PTDC/QUI-BIQ/118260/2010 to L.M.M. and PEsT-OE/EQB/LA0023/2011) and a postdoctoral grant to I.N.S.

We acknowledge David P. Speert from the University of British Columbia, Canada, for providing the two clinical isolates.

REFERENCES

 Mahenthiralingam E, Urban TA, Goldberg JB. 2005. The multifarious, multireplicon *Burkholderia cepacia* complex. Nat Rev Microbiol 3:144–156. http://dx.doi.org/10.1038/nrmicro1085.

- Lipuma JJ. 2010. The changing microbial epidemiology in cystic fibrosis. Clin Microbiol Rev 23:299–323. http://dx.doi.org/10.1128/CMR.00068 -09.
- Peeters C, Zlosnik JEA, Spilker T, Hird TJ, LiPuma JJ, Vandamme P. 2013. Burkholderia pseudomultivorans sp. nov., a novel Burkholderia cepacia complex species from human respiratory samples and the rhizosphere. Syst Appl Microbiol 36:483–489. http://dx.doi.org/10.1016/ j.syapm.2013.06.003.
- 4. Holden MT, Seth-Smith HM, Crossman LC, Sebaihia M, Bentley SD, Cerdeño-Tárraga AM, Thomson NR, Bason N, Quail MA, Sharp S, Cherevach I, Churcher C, Goodhead I, Hauser H, Holroyd N, Mungall K, Scott P, Walker D, White B, Rose H, Iversen P, Mil-Homens D, Rocha EP, Fialho AM, Baldwin A, Dowson C, Barrell BG, Govan JR, Vandamme P, Hart CA, Mahenthiralingam E, Parkhill J. 2009. The genome of *Burkholderia cenocepacia* J2315, an epidemic pathogen of cystic fibrosis patients. J Bacteriol 191:261–277. http://dx.doi.org/10.1128/ JB.01230-08.
- Zlosnik JE, Hird TJ, Fraenkel MC, Moreira LM, Henry DA, Speert DP. 2008. Differential mucoid exopolysaccharide production by members of the *Burkholderia cepacia* complex. J Clin Microbiol 46:1470–1473. http:// dx.doi.org/10.1128/JCM.02273-07.

- Zlosnik JE, Costa PS, Brant R, Mori PY, Hird TJ, Fraenkel MC, Wilcox PG, Davidson AG, Speert DP. 2011. Mucoid and nonmucoid *Burkholderia cepacia* complex bacteria in cystic fibrosis infections. Am J Respir Crit Care Med 183:67–72. http://dx.doi.org/10.1164/rccm.201002-0203OC.
- Silva IN, Ferreira AS, Becker JD, Zlosnik JEA, Speert DP, He J, Mil-Homens D, Moreira LM. 2011. Mucoid morphotype variation of *Burkholderia multivorans* during chronic cystic fibrosis lung infection is correlated with changes in metabolism, motility, biofilm formation and virulence. Microbiology 157:3124–3137. http://dx.doi.org/10.1099/ mic.0.050989-0.
- Silva IN, Tavares AC, Ferreira AS, Moreira LM. 2013. Stress conditions triggering mucoid morphotype variation in *Burkholderia* species and effect on virulence in *Galleria mellonella* and biofilm formation *in vitro*. PLoS One 8:e82522. http://dx.doi.org/10.1371/journal.pone.0082522.
- Hernandez D, François P, Farinelli L, Osterås M, Schrenzel J. 2008. De novo bacterial genome sequencing: millions of very short reads assembled on a desktop computer. Genome Res 18:802–809. http://dx.doi.org/ 10.1101/gr.072033.107.
- Boetzer M, Henkel CV, Jansen HJ, Butler D, Pirovano W. 2011. Scaffolding pre-assembled contigs using SSPACE. Bioinformatics 27: 578–579. http://dx.doi.org/10.1093/bioinformatics/btq683.